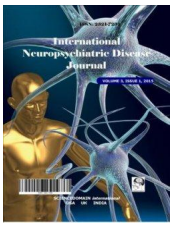

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Cerebrospinal Fluid Biomarkers of Neuroinflammation and Axonal Degeneration in Patients with Multiple Sclerosis

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Authors' contributions

This work was carried out in collaboration between all authors. Author MES designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AE and AAE managed the analyses of the study. Authors MES and AAE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Inflammation mediators have important roles in leukocyte recruitment and the central nervous system (CNS) inflammation and damage. Axonal and neuronal damage are associated with the level of CNS inflammation and determine physical handicap on multiple sclerosis (MS) patients.
Objectives: On distinguishing and inspect the associations between a group of inflammatory biomarkers {matrix metalloproteinase 9 (MMP9), neurofilament light chain (NFL), osteopontin (OPN), and chemokine ligand 13 (CXCL13)} on MS patients.
Patients and Methods: We collected the patients the electronic Mansoura Neurology department data sheet, and all patients encountered assessment by Expanded Disability Status Scale (EDSS) at the onset. All patients and control had cerebrospinal fluid (CSF) biomarkers work-up (MMP9, CXCL13, OPN, and NFL) that measured by ELISA. A correlation statistic matrix was done to demonstrate the presence of relationships between CSF biomarkers within the MS cases.
Results: The enclosed fifty patients comprising different MS subtypes {relapsing-remitting (RRMS)

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(70%), secondary progressive (SPMS) (22%) and primary progressive (PPMS) (8%)} with the mean age of 34.6±8.9 years. Healthy controls (HC) were 25. The most common clinical presentations were sensory manifestations (34%) and optic neuritis (24%). We found that the levels of CXCL13, NF-L, OPN, and MMP-9 were highly significantly increased among MS patients (P<0.0001, P<0.0001, P<0.001, and P<0.0001 respectively). The correlations between CSF biomarker levels showed a highly significant correlation between CXCL13 and MMP-9 followed by OPN and NF-L.
Conclusion: Our results showed that there is a strong association between CSF biomarkers of inflammation and axonal damage and MS, especially in RRMS patients.

Keywords: Multiple sclerosis; biomarker; expanded disability status scale.

1. INTRODUCTION

Multiple sclerosis (MS) is the most well-known neurological disease among youth, with onset at about 30 years (age range from 20 till 40). The frequency of MS is 1 per 1000 people, and the proportion of female to male patients is 1·5 to 1. The MS can be classified into three clinical subtypes according to the disease course: relapsing-remitting (RRMS; prevalence 45%), secondary progressive (SPMS; prevalence 35%), and primary progressive (PPMS; prevalence 20%) [1].

Up to now, the opinions for many autoimmune diseases, notably multiple sclerosis (MS), have yet to be characterized entirely [2]. MS is an inflammatory illness of the central nervous system (CNS) which described by a loss of myelin, gliosis and distinctive degrees of axonal and oligodendrocyte pathology forming active plaque [3,4,5,6].

Clinical disease progression results in major disability in most of MS patients [7]. The disease progression in MS can't be averted and is hard to anticipate. Biomarkers are potentially useful for predicting disease evolution and for the monitoring of therapy response, outcome in MS patients. In many neurological diseases, cerebrospinal fluid (CSF) is the body fluid of choice for screening of biomarkers since it is in close contact with the tissues, and so gives an exceptional window into existing local pathological processes [8]. While MRI mainly displays the terminal stage from the damaging mechanisms, molecular biomarkers may represent the current and accurate mechanisms and thus are relevant [9]. CSF IgG index and oligoclonal bands are used as a diagnostic tool [10]. Nevertheless, there is a lack of biomarkers for predicting and monitoring the disease progression to be used clinically [1,11].

The matrix metalloproteinases (MMPs) defined a group of zinc-dependent endopeptidases which are essential mediators of the extracellular matrix. MMPS levels increase by activation of white blood cells and behave as inflammatory immune-modulators by promoting leukocyte penetration into the CNS and moreover assist in myelin injury by the splitting of extracellular matrix proteins [12]. The increased CSF levels of MMPs, especially MMP9, are obvious in a wide spectrum of neuroinflammatory diseases, including MS [13,14,15]. In a study of MS patients with active disease, elevated levels of MMP9 were observed by ELISA in around half of the patients, which decreased by natalizumab [16].

Chemokines are considered one of the important inflammatory immune-mediators. As, leukocyte enrollment is closely controlled and participates in many exchanges among chemokine receptors, chemokines, and adhesion molecules [17]. In non-inflammatory neurological diseases, MS patients, and control volunteers, the CSF B lymphocytes showed chemokine receptors, including CXCR5 [18]. Especially, the CXCR5 ligand CXCL13 that found in active MS plaques and its level was elevated in MS patient's CSF [19,20,21]. Increased CSF concentrations of CXCL13 are associated with disease deteriorations and bad prognosis in RRMS, whereas high concentrations anticipate the conversion of the clinically isolated syndrome (CIS) into definitive MS [22,23]. CSF concentrations of CXCL13 are furthermore decreased following the therapy with high-dose from the methylprednisolone and natalizumab [24].

Osteopontin (OPN) is an immune-modulator that implicated in the inflammatory cascade that happens in MS represents complement factors and cytokines. It is a pleiotropic cytokine, found in the majority of tissues and body fluids participating in many physiological and pathological mechanisms such as malignant transformation, bone mineralization,

inflammation, atherosclerosis, and immunity [25,26]. It is believed that this pro-inflammatory cytokine plays a crucial function in the pathogenesis of MS. So it might be valuable as a biomarker. Chabas and colleagues stated that the OPN transcript is sufficiently raised in MS plaques, and anti-OPN immune-reactivity is obvious in microglia, astrocytes, macrophages, and microvascular endothelial cells within the active plaques [27]. In patients with an RRMS, CSF OPN levels increased in patients with active disease, which correlate with biomarkers of inflammation and tissue damage in the CNS [28]. Khademi and colleagues stated that the elevated CSF levels of OPN in RRMS patients were normalized after treatment with natalizumab [16].

Neurofilament-light chain (NFL) constitutes the basis to the neurofilament fiber. It is released into the CSF after axonal and neuronal injury and denoting the MS disease activity. Assessment of NFL in the CSF constitutes a valuable biomarker for the axonal damage. Their levels are increased after MS relapses and also give prognostic information [29,30,31]. Considerably, in MS patients the CSF levels of NFL decreased within six months of beginning of the natalizumab treatment, indicating both the usefulness of this therapy for MS and for follow-up of therapeutic response [32].

In the present study, we distinguished and inspected the possible associations between inflammatory biomarkers (MMP9, CXCL13, OPN, and NFL) in MS patients.

2. PATIENTS AND METHODS

This study included 50 multiple sclerosis patients recruited from the Neurology outpatient clinic of Mansoura Neurology and Neurosurgery Centre (MNNC) or admitted to the Neurology Department, Mansoura University Hospital (MUH) or follow up at Mansoura University Students Hospital (MUSH), Egypt from June 2013 to May 2014. All enrolled patients had agreed to participate in the study. All patients involved in this study were diagnosed clinically as definite MS, based on Poser et al. [33] criteria or Paty et al. [34] radiological criteria. The diagnosis of Secondary progressive MS (SPMS), Primary progressive MS (PPMS), and Relapsing Remitting MS (RRMS) was based on Lublin et al. [35] criteria. The routine laboratory investigations including CBC, blood glucose level, renal, and liver functions were within normal. We excluded any patient with a previous history of any neurological or medical diseases.

Also, 25 sex and age-matched healthy volunteers have selected as controls. None had a history of neurological, other autoimmune diseases, chronic illness, or another history of symptoms indicating demyelinating illness or family history of MS in any member. All were subjected to clinical and neurological examination and underwent a cerebral MRI. None of them had white matter lesions.

Patients were evaluated clinically using complete history taking and full neurological evaluation. We used the electronic Mansoura neurology department sheet paying concern to the onset, course, duration of illness, the precipitating factors, and either in the activity or relapse in RRMS, in evolution or plateau stage in secondary progressive MS, early signs and symptoms (sensory, kinesthetic, visual), family history of MS, other diseases, current use of corticosteroids, rehabilitation treatments, using complementary and alternative remedies. Expanded disability status scale (EDSS) is a scale that measures the function and also can objectively identify and measures the level of disability and follow-up disease progression or response to a drug. EDSS evaluates the disability in eight functional systems, cerebellar, pyramidal, and sensory, brainstem, bladder, and bowel, mental, visual, and other functions. It is 10 grades varying from 0, 0.5, 1, and 1.5 until 10. EDSS score of up to 4.5 signifies to people with MS who are fully ambulatory. Impairment defines the score of 5.0 or more in EDSS [36]. All patients had MRI Brain imaging that was conducted utilizing a 1.5 T MR machine (Symphony; Siemens AG Medical Systems, Forchheim, Germany). The MRI protocol of the brain: Axial scans were achieved with the patient supine using the standardized head coil. We used the ordinary axial T1 weighted classical spin echo TE (echo time): 14 msec, TR 540 msec. While the Axial T2 weighted was fast spin echo (TR/TE eff 3600/95; echo train length, 8) and fluid attenuation inversion recovery (FLAIR) images were carried in different planes of the brain (axial, sagittal and coronal) using of the post-contrast T1 sequence. While for post-contrast MRI study a dose of 0.1 mmol/kg of Gadolinium-diethylene-triamine-pentaacetate (Gd-DTPA) was injected via a slow intravenous route [37].

All patients and controls had a single CSF sample that obtained by performing a lumbar

puncture in a supine position at level L4-L5 under local anesthesia by using a 22 gauge 'pencil tipped' atraumatic needle (Pencan Needle B Braun) to reduce the risk of a CSF leakage as much as possible [38]. A 12 ml of CSF was gathered in an ice bath and centrifuged at 4°C for 10 min, and the supernatant was frozen and stored until analysis. Biomarker analyses were done by enzyme-linked immunosorbent assays (ELISA) via commercially existing kits for CXCL13, MMP (Quantikine Human CXCL13/BLC/BCA-1, MMP9), OPN (R&D Systems, Abingdon, UK) and NFL (Uman diagnostics, Umeå, Sweden) according to the manufacturers' instructions.

2.1 Statistical Analysis

Obtained data were presented as mean±SD, ranges, numbers, and ratios. For comparability, we used the Pearson Chi-square. P<0.05 was regarded as statistically significant. P<0.001 was considered as highly statistically significant. Mann-Whitney U test compared CSF biomarkers with subtypes of MS and healthy controls. The CSF biomarker levels in patients with MS correlations were done by Spearman's rank correlation analysis. CSF biomarker in subtypes of MS was compared by Mann-Whitney U test. The statistical analysis was conducted utilizing the SPSS (Version 15, 2006; SPSS Inc., Chicago, IL, USA) for Windows statistical package.

3. RESULTS

The study sample included 50 patients; 16 males and 34 females with the male: female ratio of 1: 2.1 and had a mean age range of 34.6±8.9 years old, range: 15-58 years, while healthy control (HC) included (n=25), eight males and 17 females with the male: female ratio 1:2.1 and had mean age range 34.2± 8.7, range: 15-58 years without statistically significant differences (Table 1).

Disease-related data showed non-significant (p>0.05) difference between males and females, apart from the significantly higher frequency of female patients and male patients were older. The most common clinical presentations were sensory manifestations (34%) then optic neuritis and pyramidal manifestations (24%). The most common MS subtypes were RRMS (70%) followed by SPMS (22%) then PPMS (8%). The mean range of EDSS was 2.2 ± 1.5 (Table 2).

All biomarkers levels were higher in MS patients, as shown in Table 3.

The CSF biomarkers correlated firmly between the two groups. The first group was CXCL13 and MMP-9 (r= 0.92, P<0.0001). While, the second group was OPN and NFL (r=0.53, P=0.0021) (Table 4).

The inflammatory biomarkers (CXCL13, MMP9, OPN, and NFL) increased significantly in all MS subtypes, most markedly in RRMS relapse patients (P<0.001) with particular importance to NFL (Table 5).

4. DISCUSSION

We used four different CSF biomarkers that were selected based on either their vital role as inflammatory immune-modulators that facilitate leukocyte entrance into the CNS causing myelin

Table 1. Demographic data of studied patients and healthy controls (HC)

Variable		Patients N=50 Mean ± SD (range) Mean± frequency (if %)	HC N=25 Mean ± SD (range) Mean± frequency (if %)
	Total	34.6±8.9 (15-58)	34.2± 8.7 (15-58)
Age (years)	**Female**	34.1± 9.3 (15-55)	33.1± 9.8 (15-55)
	Male	33.9 ± 9.3 (19-58)	35.9 ± 9.1 (19-58)
Gender ratio	**Male: Female**	1:2.1	1:2.1
	Male/Female	16/34	8/17
Age by subgroups	**10-19**	2 (4%)	1 (4%)
	20-29	16 (32%)	9 (36%)
	30-39	18(36%)	8 (32%)
	40-49	10 (20%)	5 (20%)
	50-59	4 (8%)	2 (8%)

SD= Standard deviation

Table 2. Clinical characters of MS patients

Age of onset	**Total**	27 ± 7.8 (15-50)
Mean ± SD (range)	**Male**	27± 9.8 (15-50)
	Female	26.5 ± 9.1 (18-45)
Clinical presentation	**Sensory**	17 (34 %)
Number (Percentage)	**Optic neuritis**	12(24%)
	Pyramidal	12(24%)
	Brain stem	4 (8%)
	Cerebellar	3 (6%)
	Spinal cord	2(4%)
EDSS at onset	**2.2 ± 1.5 (0-9)**	
Mean ± SD (range)		
Types of MS	**RRMS**	35 (70%)
Number (Percentage)	**SPMS**	11 (22%)
	PPMS	4 (8%)

SD= Standard deviation

Table 3. CSF biomarker levels of patients and HC

Biomarker	**Patients**		**HC**		**Test**
	Median	**Range**	**Median**	**Range**	**P-value**
CXCL13 (pg/ml)	6.8	3.9-485.2	3.9	3.9-3.9	P<0.0001
MMP-9 (ng/ml)	0.167	0.156-5.757	0.156	0.156-0.156	P<0.0001
OPN (ng/ml)	229.5	45.1-529.2	126.9	76.1-234.9	P<0.001
NF-L (ng/l)	1506.3	268.6-10107.3	413.9	101.0-852.9	P<0.0001

Table 4. Interrelation of CSF biomarkers levels in patients with MS

	CXCL13	**MMP-9**	**OPN**	**NF-L**
CXCL13	1			
MMP-9	**r= 0.92 (p<0.0001)**	1		
OPN	r=0.13 (p=1)	r=0.19 (p=1)	1	
NF-L	r=0.33 (p=0.2352)	r=0.31 (p=0.3991)	**r=0.53 (p=0.0021)**	1

Table 5. CSF biomarkers in subtypes of MS

Variable (Number)	**CXCL13**	**MMP-9**	**OPN**	**NF-L**	**Testing by Mann-Whitney U test.**
Control (20)	3.9	0.156	126.9	413.9	
RRMS (35)	25	0.61	260	1591	P<0.001
SPMS (11)	10	0.3	219	599	P<0.001
PPMS (4)	3.9	0.35	246	567	P<0.001

damage or considered as a marker of axonal injury [12,19,20,21,27,29]. Despite a considerable degree of difference, the importance of inflammatory activity seems to be greatest in MS patients with acute axonal damage by the measurement of the NFL in the CSF.

A recent panel of the CSF biomarkers; CXCL13, IL12p40, and IL–8 has been investigated as indicating active intrathecal inflammation [21]. This information is in line with the present study, especially CXCL13 and the previous reports on that chemokine [19,20,22]. Prior studies into this set of MS biomarkers stated that the concentrations increased compared to cases of non-inflammatory neurological conditions, in contrast to this study [39].

Kuhle and colleagues suggest a beneficial effect of NFL as a quantitative biomarker in MS patients for axonal injury and for monitoring the fingolimod response. This information is in line with the present study, especially in RRMS [40].

Kivisäkk and colleagues stated that their data do not aid a role for circulating OPN levels as a biomarker for disease potency in a

heterogeneous clinical manifestation, without excluding its potential role in the CSF, in control in a comparative research study such as a clinical trial, or in concert with other biomarkers. This information is not in line with the present study [41] while recently Stilund and colleagues stated that the biomarker panel including OPN showed distinctive patterns for each patient group and could be a useful tool for clinical diversification of MS subgroups. Especially in patients with PPMS were revealed to have significantly increased levels of both degenerative and inflammatory markers [42]. This is in line with the present study except for the most critical MS subtype which was PPMS while in our study it was RRMS. This may be due to the small number of PPMS in our research, and this needs more numbers of PPMS patients' in the future investigational research.

5. STUDY LIMITATIONS

Regarding the small sample size, the presenting data are for the MS patients who approved to be in the study during this period. Regarding the MS definition criteria, although we used the old diagnostic criteria these did not affect our results as it is the same MS patient in old or new criteria. Regarding the ideas for new researches and statistics like MS treatments and their potential impact on the MS biomarkers, these will be in the future studies. As the patients just started the medications we cannot assess the responses. These need long duration, big sample size, good registry for the patients and their follow up. These will need another specific study design.

6. CONCLUSIONS

Our results showed that there is a strong association between CSF biomarkers of inflammation and axonal damage and MS, especially in RRMS patients.

CONSENT

As per international standard or university standard written patient consent has been collected and preserved by the authors.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Teunissen CE, Dijkstra C, Polman C. Biological markers in CSF and blood for axonal degeneration in multiple sclerosis. Lancet Neurol. 2005;4(1):32-41.
2. Bach JF, Chatenoud L. Tolerance to islet autoantigens in type 1 diabetes. Annu Rev Immunol. 2001;19:131–161.
3. Rubin SM. Management of multiple sclerosis: An overview. Dis Mon. 2013;59: 253–260.
4. Ozakbas S, Kaya D, Idiman E. Early onset multiple sclerosis has worse prognosis than adult onset multiple sclerosis based on cognition and magnetic resonance imaging. Autoimmune Dis. 2012;2012: 563989.
5. Lin GY, Wang CW, Chiang TT, Peng GS, Yang FC. Multiple sclerosis presenting initially with a worsening of migraine symptoms. J Headache Pain. 2013;14:70.
6. Rotondi M, Batocchi AP, Coperchini F, Caggiula M, Zerbini F, Sideri R, et al. Severe disability in patients with relapsing-remitting multiple sclerosis is associated with profound changes in the regulation of leptin secretion. Neuroimmunomodulation. 2013;20:341–347.
7. Confavreux C, Vukusic S. Natural history of multiple sclerosis: A unifying concept. Brain. 2006;129:606–616.
8. Teunissen CE, Khalil M. Neuro-filaments as biomarkers in multiple sclerosis. Mult Scler. 2012;18(5):552-556.
9. Teunissen CE, Iacobaeus E, Khademi M, Brundin L, Norgren N, Koel-Simmelink MJ, et al. Combination of CSF N-acetylaspartate and neurofilaments in multiple sclerosis. Neurology. 2009;72: 1322–1329.
10. Deisenhammer F, Egg R, Giovannoni G, Hemmer B, Petzold A, Sellebjerg F, et al. EFNS guidelines on disease-specific CSF investigations. Eur J Neurol. 2009;16:760–770.
11. Teunissen CE, Killestein J and Giovannoni G. Biomarker research in MS: Addressing

axonal damage and heterogeneity. Biomark Med. 2007;1:111–119.
12. Kieseier BC, Seifert T, Giovannoni G, Hartung HP. Matrix metalloproteinases in inflammatory demyelination: Targets for treatment. Neurology. 1999;53(1):20-25.
13. Gijbels K, Masure S, Carton H, Opdenakker G. Gelatinase in the cerebrospinal fluid of patients with multiple sclerosis and other inflammatory neurological disorders. J Neuroimmunol. 1992;41(1):29-34
14. Leppert D, Ford J, Stabler G, Grygar C, Lienert C, Huber S, et al. Matrix metalloproteinase-9 (gelatinase B) is selectively elevated in CSF during relapses and stable phases of multiple sclerosis. Brain. 1998;121(Pt 12):2327-2334.
15. Ram M, Sherer Y, Shoenfeld Y. Matrix metalloproteinase-9 and autoimmune diseases. J Clin Immunol. 2006;26(4):299-307.
16. Khademi M, Bornsen L, Rafatnia F, Andersson M, Brundin L, et al. The effects of natalizumab on inflammatory mediators in multiple sclerosis: Prospects for treatment-sensitive biomarkers. Eur J Neurol. 2009;16(4):528-536.
17. Ransohoff RM, Kivisäkk P, Kidd G. Three or more routes for leukocyte migration into the central nervous system. Nat Rev Immunol. 2003;3(7):569-581.
18. Sørensen TL, Roed H, Sellebjerg F. Chemokine receptor expression on B cells and effect of interferon-beta in multiple sclerosis. J Neuroimmunol. 2002;122(1-2): 125-131.
19. Krumbholz M, Theil D, Cepok S, Hemmer B, Kivisäkk P, Ransohoff RM, et al. Chemokines in multiple sclerosis: CXCL12 and CXCL13 up-regulation is differentially linked to CNS immune cell recruitment. Brain. 2006;129(Pt 1):200-211.
20. Kuenz B, Lutterotti A, Ehling R, Gneiss C, Haemmerle M, Rainer C, et al. Cerebrospinal fluid B cells correlate with early brain inflammation in multiple sclerosis. PLoS One. 2008;3(7):e2559.
21. Bielekova B, Komori M, Xu Q, Reich DS, Wu T. Cerebrospinal Fluid IL-12p40, CXCL13 and IL-8 as a combinatorial biomarker of active intrathecal inflammation. PLoS One. 2012;7(11): e48370.
22. Brettschneider J, Czerwoniak A, Senel M, Fang L, Kassubek J, Pinkhardt E, et al. The chemokine CXCL13 is a prognostic marker in clinically isolated syndrome (CIS). PLoS One. 2010;5(8):e11986.
23. Khademi M, Kockum I, Andersson ML, Iacobaeus E, Brundin L, Sellebjerg F, et al. Cerebrospinal fluid CXCL13 in multiple sclerosis: A suggestive prognostic marker for the disease course. Mult Scler. 2011; 17(3):335-343.
24. Sellebjerg F, Börnsen L, Khademi M, Krakauer M, Olsson T, Frederiksen JL, et al. Increased cerebrospinal fluid concentrations of the chemokine CXCL13 inactive MS. Neurology. 2009;73(23): 2003-2010.
25. Mazzali M, Kipari T, Ophascharoensuk V, Wesson JA, Johnson R, Hughes J. Osteopontin- a molecule for all seasons. QJM. 2002;95(1):3-13.
26. Wang KX, Denhardt DT. Osteopontin: role in immune regulation and stress responses. Cytokine Growth Factor Rev. 2008;19(5-6):333-345.
27. Chabas D, Baranzini SE, Mitchell D, Bernard CC, Rittling SR, Denhardt DT, et al. The influence of the proinflammatory cytokine, osteopontin, on autoimmune demyelinating disease. Science. 2001; 294(5547):1731-1735.
28. Börnsen L, Khademi M, Olsson T, Sørensen PS, Sellebjerg F. Osteopontin concentrations are increased in cerebrospinal fluid during attacks of multiple sclerosis. Mult Scler. 2011;17(1): 32-42.
29. Lycke JN, Karlsson JE, Andersen O, Rosengren LE. Neurofilament protein in cerebrospinal fluid: A potential marker of activity in multiple sclerosis. J Neurol Neurosurg Psychiatry. 1998;64(3):402-404.
30. Norgren N, Sundström P, Svenningsson A, Rosengren L, Stigbrand T, Gunnarsson M. Neurofilament and glial fibrillary acidic protein in multiple sclerosis. Neurology. 2004;63(9):1586-1590.
31. Salzer J, Svenningsson A, Sundström P. Neurofilament light as a prognostic marker in multiple sclerosis. Mult Scler. 2010; 16(3):287-292.
32. Gunnarsson M, Malmeström C, Axelsson M, Sundström P, Dahle C, Vrethem M, et al. Axonal damage in relapsing multiple sclerosis is markedly reduced by natalizumab. Ann Neurol. 2011;69(1):83-89.
33. Poser CM, Paty DW, Scheinberg L, McDonald WI, Davis FA, Ebers GC, et al.

New diagnostic criteria for multiple sclerosis: Guidelines for research protocols. Ann Neurol. 1983;13:277-231.
34. Paty D and Li D. Diagnosis of MS: Do we need new diagnostic criteria? In Siva A, Kesselring J, Thompson A, ed. Frontiers in MS Martin Dunitz. 1998;2:47-50.
35. Lublin FD, Reingold SC. Define the clinical course of multiple sclerosis: Results of an international survey. Neurology. 1996;46: 907-911.
36. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: An expanded disability status scale (EDSS). Neurology. 1983;33(11):1444-1452.
37. Simon JH, Li D, Traboulsee A, Coyle PK, Arnold DL, Barkhof F, et al. Standardized MR imaging protocol for multiple sclerosis: Consortium of MS Centers consensus guideline. AJNR Am J Neuroradiol. 2006; 27(2):455-461.
38. Shen DD, Artru AA, Adkison KK. Principles and applicability of CSF sampling for the assessment of CNS drug delivery and pharmacodynamics. Adv Drug Deliv Rev. 2004;56(12):1825-1857.
39. Romme Christensen J, Börnsen L, Khademi M, Olsson T, Jensen PE, Sørensen PS, et al. CSF inflammation and axonal damage are increased and correlate in progressive multiple sclerosis. Mult Scler. 2013;19(7):877-84.
40. Kuhle J, Disanto G, Lorscheider J, Stites T, Chen Y, Dahlke F, et al. Fingolimod and CSF neurofilament light chain levels in relapsing-remitting multiple sclerosis. Neurology. 2015;84(16):1639-1643.
41. Kivisäkk P, Healy BC, Francois K, Gandhi R, Gholipour T, Egorova S, et al. Evaluation of circulating osteopontin levels in an unselected cohort of patients with multiple sclerosis: Relevance for biomarker development. Mult Scler. 2014;20(4):438-444.
42. Stilund M, Gjelstrup MC, Petersen T, Møller HJ, Rasmussen PV, Christensen T. Biomarkers of inflammation and axonal degeneration/damage in patients with newly diagnosed multiple sclerosis: Contributions of the soluble CD163 CSF/serum ratio to a biomarker panel. PLoS One. 2015;10(4): e0119681.

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